Virology
Research Center
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Diagnostic Virology after Organ Transplantation

Seyed Alireza Nadji, Ph.D.

VRC, NRITLD

Viral infection in Transplantation

- Perturbations of immunity can influence the pathogenesis of viral infections
  - Prominent among these: many herpesvirus infections in immunocompromised hosts.
    - Herpes simplex, CMV, EBV, VZV, HHV-6 human herpesvirus 6, HHV- 7 human herpesvirus 7
  - Polyomavirus type BK and JC virus.
  - Adenovirus, Influenza, hMpv, RSV, Parainfluenza, Rhinovirus.

Diagnosis

- Diagnosis of viral infections in immunocompromised patients can be difficult.
  - Total and differential leukocyte counts tend to be unhelpful,
  - clinical signs and symptoms of infection are often atypical,
  - and symptoms produced by the host's immune response may be absent.

Diagnosis

- Serology has little role in diagnosis;
  - patients with dysfunctional immunity do not usually mount appropriate immune responses rapidly enough to influence decisions about management.
- The mainstay of diagnosis is detection of virus in appropriate clinical specimens;
  - rapid procedures, such as direct antigen detection, nucleic acid detection and culture amplified enzyme immunoassays
- In post-transplantation, regular surveillance may be helpful;
  - virus shedding may precede the onset of symptoms, and initiation of therapy as soon as virus is detected may prevent extensive tissue damage.

HSV

- The frequency and severity of recurrent episodes of herpes simplex are increased in patients with allograft recipients
  - commonly presents in the second week after transplantation.
- Major manifestations; pneumonia and hepatitis.
- Virus detection is the mainstay of laboratory diagnosis.
  - To diagnose HSV pneumonia, bronchoalveolar lavage fluid or, preferably, a lung biopsy specimen is necessary.
  - Virus culture; can take up to 7 days before a characteristic cytopathic effect (CPE) develops
  - More rapid procedures; direct detection of viral antigens and culture-amplified enzyme immunoassays.

  however, PCR detection of viral DNA is gaining increased acceptance

CMV

- Primary infection is acquired at any time throughout life;
  - by 40 years of age, 50–70 percent of the general population are infected.
- Reactivation is detected in the 2nd and 3rd months after transplantation.
- End-organ disease attributed to CMV includes (prominent among) *pneumonitis, gastrointestinal lesions, hepatitis.*

CMV diagnosis

- Diagnosis of CMV disease requires detection of virus in material from the affected organ
  - CMV pneumonitis; detection of CMV in bronchoalveolar lavage fluid or transbronchial biopsy detection of CMV in blood by pp65 antigenemia or by PCR.

CMV diagnosis

- In patients with neurologic manifestations of cytomegalovirus infection (including chorioretinitis) and gastrointestinal disease (colitis and gastritis, often with ulceration)
  - Blood-based cytomegalovirus assays may be negative.
    - Thus, invasive procedures such as colonoscopy with biopsy or lumbar puncture may be necessary

CMV diagnosis

- Among immunocompromised patients, serologic methods are not useful
- Shedding of CMV is very common in patients with impaired cell-mediated immunity
  - CMV shedding in urine or saliva is of limited value.

Quantitation of CMV in blood

- The most valuable laboratory diagnostic tests are those that quantitate CMV in blood
  - greater quantity of virus (viral DNA) in whole blood or in plasma correlates with greater risk of CMV disease.

Threshold quantities

- Difficult to make recommendations regarding threshold quantities of CMV that will predict disease
  - Many different approaches are used,
    - pp65 antigenemia, quantitative competitive PCR, NASBA for pp67 mRNA, hybrid capture, and real-time PCR;
      - commercial kits are available for some of these and a number of institutions use in-house PCR or antigenemia assays.
  - More complicated because thresholds for predicting disease differ according to the clinical setting.
    - among HCT recipients, lower levels of CMV pp65 antigen-positive cells or of CMV DNA in blood are predictive of CMV disease than in solid organ transplant recipients

	CMV DNAemia copy/ml		CMV Antigenemia pp65 positive WBC /2.5*10^5 PB leukocytes	
	Infection	Disease	Infection	Disease
SOT Commercial quantitative PCR assay	1660 Plasma	5000 Plasma	16	170
in-house quantitative PCR system	10000 Whole Blood	300000 Whole Blood	1	125
Renal transplant Commercial quantitative PCR	375 Whole Blood	19650 Whole Blood	25	500
Real-time PCR assay	125,000 CMV DNA copies per 2 * 10^5 PBL		12	
Real-time PCR assay	130 or more genome equivalents per 2 * 10^5 PBL		1 or more pp65 positive cell	

Clinicians/Laboratories

- Until a standard approach to quantitation of CMV in blood is widely accepted;
  - clinicians to work closely with their clinical laboratories to determine locally the levels of antigenemia or CMV DNAemia that are correlated with disease occurrence
  - the threshold levels will vary among different categories of transplanted patients

ADENOVIRUS

- Not nearly as prevalent as the herpesviruses , adenoviruses have been isolated from transplant recipients, and have contributed to their morbidity and mortality.
- isolation of adenovirus from stool, throat, urine and peripheral blood.
  - The virus often appears in the blood about 3 weeks following the transplant
  - Pneumonia, gasteroenteritis and hepatic abnormalities
  - Adenovirus species A, B, C (78% of all positive cases), D, and F.
    - Ad1, 2, 4, 5, 6, 11,31, 34, and 35 .

Adenovirus
Diagnostic approach

- The site of infection should determine the type of sample to test;
  - STOOL; Gasterointestinal disease
  - Urine; Genitourinary disease
  - Respiratory samples; Pneumonia
- Once disease is documented, Quantitative Viral load test of blood should be consider as a marker for monitoring disease progression and treatment response.
- Various diagnostic techniques have been described:
  - Serology, Antigen detection, Culture, Nucleic acid testing, Pathology

Adenovirus
Diagnostic approach

- Culture; traditional gold standard!
  - Time consuming; several days to weeks
  - Rapid shell vial test; reduced sensitivity.
- PCR is more sensitive
  - More increasingly use; the ease and sesitivity
  - Applied to whole blood as a significant screening method;
    - Documented in pediatric HSCT recipients, not in adults.
- No specific threshold quantity for prediction the disease
  - Higher viral DNA levels($>10^6$ copies/ml) associated with greater risk of death among pediatric transplant recipients.

ADENOVIRUS …

- Adenoviraemia is commonly found among adult SOT recipients and does not predict disease,
  - Should not be used to prospectively screening the disease
    - Possible exception of small bowel transplant recipients.

Dynamic trends in adenovirus load in blood also appear to be useful tool in monitoring response to therapy.

ADENOVIRUS …

- In summary from the various transplant studies
  - PCR or real-time PCR is an effective and sensitive method to detect adenovirus in clinical specimens.
  - Detection of adenovirus in stool and throat swab is usually not associated with adenovirus disease.
  - Detection of adenovirus in the peripheral blood is more often associated with disease, but only very roughly about half the time.
  - The virus often appears in the blood about 3 weeks before the onset of symptoms
    - virus DNA levels in the blood of greater than 10^6 to 10^7 (or more) copies/mL pose an increased risk for fatal outcome.
  - Pediatric patients are more at risk for adenovirus
    - having higher viral load

ADENOVIRUS …

- Recommended the use of real-time PCR to monitor adenovirus in the blood
  - successive times following transplantation,
  - especially in T cell- depleted grafts,
  - looking for an increase in viral load and, if possible, withdrawal of immunosuppression.
- Anecdotal evidence suggests that treatment with ribavirin and cidofovir to suppress adenovirus may be beneficial.

Polyomavirus infections

- BKV and JCV are commonly detected in the urine and blood of transplant patients, suggesting that immunosuppression allows latent infections to reactivate.
  - Infections are frequent in the second month after renal transplantation, but late infections, occurring several months or even years later, are not unusual.
  - BKV is most closely associated with kidney disease or cystitis in allograft recipients, either kidney transplant or BMT recipients

BKV Diagnosis

- By demonstrating characteristic intranuclear inclusions in exfoliated urinary epithelial cells and Polyomavirus antigens within these cells can be confirmed by immunofluorescence.
- The use of single primed or nested PCR technique has greatly increased the sensitivity of assays designed to identify BKV in blood and urine of such patient populations.
  - Increasingly common is the screening of clinical samples for BKV since PCR methods became widely available
- Because the population in general already possess antibody titers to both BKV and JCV, serology has not been useful in assessing the importance of BKV in suspected infections

Diagnosis of Respiratory Viral Infection

- By combination of SEROLOGY, NUCLEIC ACID TESTING, and Histopathology.
- SEROLOGY
  - In general not useful for initial diagnosis.
  - Reduced sensitivity among transplant recipients.
- Virus isolation
  - Available for most of common RNA viruses except hMPV and Coronaviruses.
  - Special cell line and condition needed to grow the viruses.
  - Tend to be inefficient;
    - Depends on site of sampling
      - BAL and Nasal wash; greatest yield
  - Time consuming; dependent on virus, viral inoculums, cell line and growth condition from 3 –21 days.

Diagnosis of Respiratory Viral Infection

- Virus isolation cont..
  - Shell vial assay;
    - More rapid, earlier detection (24–48h)
    - Lower sensitivity compared to traditional culture method.
- Rapid antigen detection
  - Despite their speed (30–60 min);
    - Substantially lower sensitivity among immunocompromised patients specially adult.
      - RSV; 15% for nasal wash – 89% for BAL.
  - Direct fluorescence assay (DFA)
    - Limited by lack of reagents for some of viruses (hMPV, Rhino & Corona)
    - Appear to be less sensitive than PCR
- PCR-based Assay;
  - appear to be the most sensitive diagnostic tools available and most allow for simultaneous detection of a broad rang of respiratory pathogens from single sample.

More examples of why you need clinical virologists to interpret results

CMV in sputum: may not be at all important unless also found in blood

adeno in blood: probably not requiring treatment unless increases by 1-2 logs in a day or until log 6

BK in urine: unimportant in renal transplants because so common - becomes important once in blood

HSV in blood: you would think it is important, but sometimes it is just a marker of high mortality risk

Thanks for your patience

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